

# Fly Reservoir Associated with *Wohlfahrtiimonas* Bacteremia in a Human

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*Wohlfahrtiimonas* species bacteria were isolated from the bloodstream of a patient with septicemia and wound myiasis. Environmental investigations identified a *Wohlfahrtiimonas* sp. among insects in the Americas and in a previously undescribed vector, the green bottle fly (*Lucilia sericata*). The isolates possibly represent a new species within the genus *Wohlfahrtiimonas*.

*Wohlfahrtiimonas chitiniclastica* is a rarely reported cause of bacterial infection that has been isolated in humans and other mammals from a variety of organs (online Technical Appendix Table, <https://wwwnc.cdc.gov/EID/article/24/2/17-0913-Techapp1.pdf>). In addition, *Wohlfahrtiimonas* spp. have been isolated from 4 species of nonbiting flies in Asia and Europe (1–4) that can cause myiasis, fly larvae infestation of a host's tissue. Wound myiasis has been reported in patients infected with *W. chitiniclastica* and with *Ignatzschineria* spp., an organism closely related to *W. chitiniclastica* (online Technical Appendix Table). These findings provide evidence that *W. chitiniclastica* is transmitted by flies or fly larvae during myiasis. However, no reported attempt has been made to isolate *Wohlfahrtiimonas* spp. or *Ignatzschineria* spp. from larvae associated with a patient. We report a case of *Wohlfahrtiimonas* infection in a man in Washington, USA, and results of environmental investigations.

## The Study

The case-patient was a 57-year-old man who developed wet gangrene of the right ankle and myiasis below the

waist. Hematology at hospital admission was notable for leukocytosis and a predominance of neutrophils with a high ratio of band neutrophils (online Technical Appendix). Chronic cirrhosis, localized lung atelectasis, and multiorgan failure secondary to septic shock were diagnosed. The patient underwent amputation below the right knee but died 3 days after admission. Blood, urine, and tracheal aspirates collected <8 hours after admission revealed a mixed bacterial infection, including gram-positive cocci and gram-negative rods (online Technical Appendix). *Propionibacterium acnes* and *Staphylococcus hominis* ssp. *hominis* were isolated from blood cultures, in addition to an unidentifiable gram-negative rod. No medical history was available; proxy interviews excluded recent travel outside Washington.

We performed presumptive identification of the gram-negative rod with phenotypic studies and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (online Technical Appendix). Amplification and sequencing of the near full-length 16S ribosomal RNA (rRNA) gene was performed, a phylogenetic tree was inferred by using the neighbor-joining method, and the topology was assessed by a bootstrap analysis of 1,000 replicates (online Technical Appendix). We used pulsed-field gel electrophoresis (PFGE) to assess isolate relatedness (online Technical Appendix).

Because larvae found on the patient had been discarded, we collected live and dead insects from the patient's home and identified them to genus or species level (online Technical Appendix). To remove surface contamination, all live fly larvae and adult specimens were rinsed 5 times with sterile phosphate-buffered saline (PBS), homogenized, and sequentially diluted. We cultured the first rinse, fifth rinse, and diluted homogenates to isolate *Wohlfahrtiimonas* spp. (online Technical Appendix).

We identified 6 species of flies (online Technical Appendix) and collected live larvae (≈20) from the patient's house (Table, batch 1). We performed bacterial culture on a pooled sample of half of these larvae (Table, sample 2) and then individually on adult flies that emerged from the other half (Table, samples 3–5). One green bottle fly (*Lucilia sericata*) (Figure 1) was caught alive in the house in a sterile container and laid eggs inside the container before dying (Table, batch 2). We isolated a *Wohlfahrtiimonas* sp. from 2 of 6 insect samples on blood agar plates (Table, samples 2–7) but not from any other samples,

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**Table.** Culture results for *Wohlfahrtiimonas* spp. from samples collected from patient with septicemia and wound myiasis and the patient's home, Washington, USA\*

Collection batch no.	Sample no.	Specimen	Specimen description	Culture <i>Wohlfahrtiimonas</i> spp.
NA	1	Blood	Isolate sent to public health laboratories from admitting hospital	Aerobic growth on blood agar (isolate 22912)
1	2	Fly larvae (unidentified species, n ≈ 20)	Larvae collected from underneath carpet where patient was found	Growth on diluted homogenate on blood agar at 25°C (isolate 22913)
1	3	House fly ( <i>Musca domestica</i> )	Emerged from larvae of sample no. 2	No growth
1	4	Unidentified species in the family Calliphoridae	Emerged from larvae of sample no. 2	No growth
1	5	<i>Calliphora vicina</i>	Emerged from larvae of sample no. 2	No growth
2	6	Green bottle fly and eggs ( <i>Lucilia sericata</i> )	Green bottle fly caught inside the patient's home and laid eggs inside a sterile container	Not cultured
2	7	<i>Lucilia sericata</i> larva	Larva obtained from the egg in batch no. 2	Growth on fifth wash (isolate 22914) and diluted homogenate (isolate 22915) on blood agar at 25°C
NA	8	Meat and fruit	Fed to flies from samples 3–7 were extracted	No growth

\*NA, not applicable

including adult flies that emerged from the positive batch of larvae.

The isolates grew on blood agar, yielding colonies with a smooth center and rough edges, and displayed  $\alpha$  hemolysis. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry yielded a presumptive result of *W. chitiniclastica* (online Technical Appendix). A phylogenetic analysis of the 16S rRNA gene sequence of all isolates (1,462 bp; see Figure 2 for GenBank accession numbers) showed that the most closely related type strains were *W. chitiniclastica* DSM 18708<sup>T</sup> (98.3% sequence similarity) and *W. larvae* JCM 18424<sup>T</sup> (97.3% sequence similarity) (Figure 2). The PFGE pattern indicated that all isolates from flies and fly larvae were indistinguishable and 74% similar to that of the patient isolate (online Technical Appendix).

## Conclusions

Our isolates possibly represent a new species in the genus *Wohlfahrtiimonas* based on the percentage sequence similarity with *W. chitiniclastica* and *W. larvae* type strains (5). We isolated *Wohlfahrtiimonas* sp. from insects in the Americas and in a previously undescribed host, the green bottle fly (*L. sericata*, Diptera: Calliphoridae). Previously, *Wohlfahrtiimonas* spp. have been identified in only 4 species of flies in Asia and Europe (*Wohlfahrtia magnifica*, *Chrysomya megacephala*, *Hemeta illucens*, *Musca domestica*) (1–4), each representing a different fly family (Diptera: Sarcophagidae, Calliphoridae, Stratiomyidae, and Muscidae, respectively). We isolated a *Wohlfahrtiimonas* sp. from a larva hatched from eggs laid by a fly in a sterile container, providing evidence that *Wohlfahrtiimonas* can be transmitted vertically.

*L. sericata* has been associated with *W. chitiniclastica* infection in a patient with myiasis and bacteremia

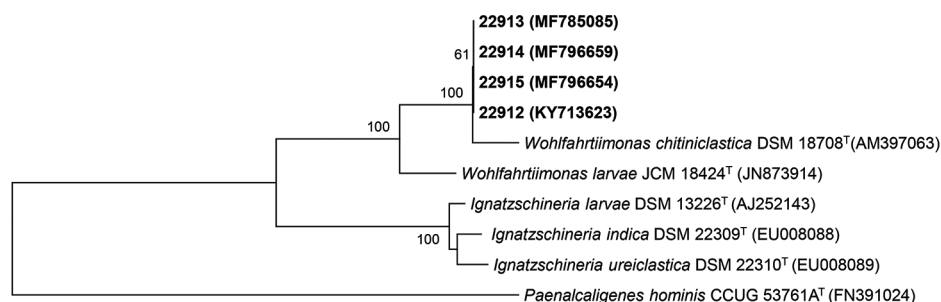
only once, in the United Kingdom (6), but a definitive link could not be established in that case because larvae from the patient had been discarded. The scarcity of reports of *Wohlfahrtiimonas* spp. infections might be attributable to the difficulty in laboratory identification (7,8) or because wound myiasis is routinely addressed with broad-spectrum antimicrobial drugs.

Because the pooled larvae (Table, sample 2) emerged as multiple fly species, we are unable to ascertain in which other species *Wohlfahrtiimonas* sp. growth occurred. *Wohlfahrtiimonas* was not isolated from these adult flies, which



**Figure 1.** Green bottle fly (*Lucilia sericata*), caught inside home of patient with septicemia and wound myiasis in Washington, USA. The fly laid eggs inside a sterile container, and *Wohlfahrtiimonas* spp. were isolated from a larva hatched from these eggs. Photo courtesy of T. Whitworth.

**Figure 2.** Neighbor-joining phylogenetic tree of 16S rRNA gene sequences of *Wohlfahrtiimonas* spp. isolate from a patient with septicemia and wound myiasis in Washington, USA (laboratory identification no. 22912), isolates from flies and fly larvae (laboratory identification nos. 22913, 22914, 22915), and the most closely related type strains. Numbers at nodes denote bootstrap percentages based on 1,000 replicates; only values >50% are shown. GenBank accession numbers are given in parentheses. Bold indicates strains isolated in this study. The tree was rooted with *Paenalcigenes hominis* CCUG 53761A<sup>T</sup> as the outgroup. Scale bar indicates substitutions per nucleotide position.



might be because the competent host was not present in the batch of larvae left to emerge or because of the association between *Wohlfahrtiimonas* spp. and flies during successive developmental stages. Indeed, previous studies isolated *W. chitiniclastica* from the gut of larvae and adult flies (1,3,4), and *Ignatzschineria* spp. are hypothesized to play a role in larval development (9), indicating that these bacteria might belong to fly microbiota. In one study, the relative abundance of *Ignatzschineria* spp. fluctuated during life stages of *L. sericata* and was among the dominant bacterial genera during the larval and pupal life stages (10). Bacterial flora further decline during pupation, when reorganization of the intestinal tract leads to extrusion of the gut lining (11). These factors might explain why we did not isolate *Wohlfahrtiimonas* spp. from the adult flies that emerged, or alternatively, our protocol might have been of insufficient diagnostic sensitivity to detect *Wohlfahrtiimonas* spp. among adult flies.

The concurrent isolation of *Wohlfahrtiimonas* sp. from the blood of a patient with myiasis and from fly larvae found at the patient's home provides further evidence that fly larvae can act as vectors of *Wohlfahrtiimonas* spp. Because PFGE patterns of the isolates obtained from the fly larvae and from the patient's blood did not match, we cannot definitively identify the fly species that led to his infection.

We isolated *Wohlfahrtiimonas* sp. from a patient's blood along with other bacteria, precluding us from assessing the pathogenicity of our isolate. However, in 2 previous reports (12,13), *W. chitiniclastica* was the only bacterium isolated from the blood, indicating its pathogenic potential (online Technical Appendix Table 1).

Most cases of *W. chitiniclastica* infection have occurred among persons with a history of poor hygiene and exposed wounds (online Technical Appendix Table 1). Green bottle flies are among the most common species associated with myiasis in the United States (14), and risk for infection is expected during warm environmental conditions favorable to their development. In addition, green bottle fly larvae are the most commonly used larvae for

maggot debridement therapy (15). Infection with *Wohlfahrtiimonas* spp. should be considered as a potential risk for patients undergoing this therapy.

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## About the Author

Dr. Bonwitt is a veterinarian and Epidemic Intelligence Service officer assigned to the Washington State Department of Health. His research interests are zoonotic and emerging infectious diseases and qualitative research at the animal-human interface.

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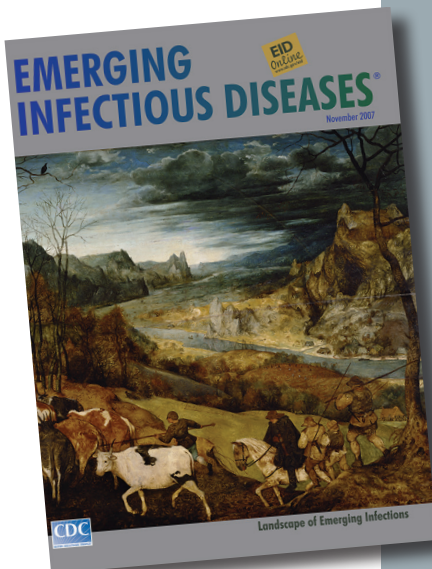
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# etymologia revisited

## Tularemia

[tü-lə-rē-mē-ə]



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in November 2007**

An infectious, plaguelike, zoonotic disease caused by the bacillus *Francisella tularensis*. The agent was named after Tulare County, California, where the agent was first isolated in 1910, and Edward Francis, an officer of the US Public Health Service, who investigated the disease. Dr. Francis first contracted “deer fly fever” from a patient he visited in Utah in the early 1900s. He kept a careful record of his 3-month illness and later discovered that a single attack confers permanent immunity. He was exposed to the bacterium for 16 years and even deliberately reinfected himself 4 times.

Tularemia occurs throughout North America, many parts of Europe, the former Soviet Union, the People’s Republic of China, and Japan, primarily in rabbits, rodents, and humans. The disease is transmitted by the bites of deerflies, fleas, and ticks; by contact with contaminated animals; and by ingestion of contaminated food or water.

Clinical manifestations vary depending on the route of introduction and the virulence of the agent. Most often, an ulcer is exhibited at the site of introduction, together with swelling of the regional lymph nodes and abrupt onset of fever, chills, weakness, headache, backache, and malaise.

**Sources:** Dorland’s illustrated medical dictionary, 31st edition. Philadelphia: Saunders; 2007; Benenson AS, editor. Control of communicable diseases manual. Washington: American Public Health Association; 1995; [www.whonamedit.com](http://www.whonamedit.com)

# Fly Reservoir Associated with *Wohlfahrtiimonas* Bacteremia in a Human

## Technical Appendix

### One Case Presentation

The case-patient's hematology upon admission was notable for leukocytosis 21.8 K/ $\mu$ L (reference 3.8–11.0 K/ $\mu$ L) and a predominant neutrophil/polymorphonuclear neutrophil ratio of 44.0% (reference 38.0%–70.0%) with a high ratio of band neutrophils, 33.0% (reference 0%–9.0%).

Blood, urine, and tracheal aspirates collected <8 hours after admission revealed a mixed bacterial infection, including gram-positive cocci and gram-negative rods. Blood culture yielded *Propionibacterium acnes* (field draw, anaerobic bottle), *Staphylococcus hominis* ssp. *hominis* and *Wohlfahrtiimonas* sp. (short draw, aerobic bottle only). Tracheal aspirates yielded heavy-growth *Haemophilus influenzae*. The urine culture was negative. *Wohlfahrtiimonas* sp. could not be identified by the referring hospital and the samples were sent for identification to the Reference Bacteriology Laboratory at the Washington State Public Health Laboratories (PHL).

### Two Methods

#### Phenotypic Studies

The isolate obtained from the patient was inoculated on blood agar, MacConkey agar, and heart infusion tyrosine agar at 35°C in aerobic conditions. Phenotypic studies for presumptive identification were performed as previously described (*1*). The isolate was tested for anaerobic growth at 35°C by using an anaerobic gas pack (BD GasPak, Becton Dickinson, Franklin Lakes, NJ, USA) in an anaerobic jar.

#### MALDI-TOF MS

Isolates were initially analyzed by matrix-assisted laser desorption/ionization time-of-flight mass-spectrometry (MALDI-TOF MS, MALDI Biotyper CA System, Bruker Daltonics,

Billerica, MA, USA) in accordance with the manufacturer's recommendations (2). The generated spectra were compared with the spectra referenced in the MALDI-TOF MS database (PHL: RUO library MBT BD-5627 MSP List; CDC: Bruker 6903 database). For analysis, an isolate was considered as identified for log scores >2.0.

### **16S Ribosomal RNA Sequencing**

Purification of whole-cell DNA and amplification and sequencing of the near full-length 16S ribosomal RNA (rRNA) gene were performed at CDC as previously described (3). The 16S rRNA gene sequence was assembled with Geneious 8.1.8 software (Biomatters, Auckland, New Zealand) by using de novo assembly. To identify related gene sequences in the GenBank database, consensus sequence was submitted to GenBank by using BLASTN software (<https://www.ncbi.nlm.nih.gov/blast/>). A multiple sequence alignment was created by using Clustal W (within Geneious 8.1.8), from which gaps and 5' and 3' ends were trimmed.

We inferred a phylogenetic tree by using the neighbor-joining method (4), and the topology was assessed by a bootstrap analysis of 1000 replicates (5). We computed the evolutionary distances by using the Tamura-Nei method (6); they are in the units of the number of base substitutions per site. Evolutionary analyses were conducted in MEGA6 (7).

### **Entomology**

All larvae found on the patient were discarded before the investigation and could not be sampled, but live and dead insects were collected from the patient's home. Insects were identified to genus or species level by using standard entomological identification keys (8–10). Live fly larvae were left to hatch at room temperature inside a mosquito-breeding container (BioQuip Products, Rancho Dominguez, CA, USA). Fly larvae were provided raw meat and freshly boiled chicken; adult flies were provided slices of fresh fruit.

### **Bacterial Isolation from Insects**

Live fly larvae and adults were cultured for *Wohlfahrtiimonas* spp. Because the preferred growth medium of *Wohlfahrtiimonas* spp. is poorly described, all samples were inoculated on 3 replicates of blood agar, MacConkey agar, and nutrient agar. Phenylethyl alcohol agar was used for initial specimens (larvae) obtained from the patient's home. Plates were incubated at 25°C, 30°C, and 35°C in aerobic conditions over 2 days.

To remove surface contamination, we rinsed all insect specimens 5 times with sterile PBS in sterile tubes, as described previously (11). We homogenized the washed specimens with a sterile mortar (Fisher Scientific, Richardson, TX, USA) in 0.5 mL PBS and sequentially diluted to 1/1000. The first wash, fifth wash, undiluted homogenate, and diluted homogenate were inoculated in the same manner as fly larvae and adults. The objective was to verify whether surface contamination was present (first wash) and, if so, whether it was removed through rinsing (fifth wash). To verify that live insects were not inoculated with *Wohlfahrtiimonas* spp. through contaminated food, the meat and fruit (with the exception of boiled chicken) was homogenized and cultured in the same manner as fly larvae and adults.

To reduce the risk for agar plates being overgrown by other bacterial species and increase the sensitivity of isolating *Wohlfahrtiimonas* spp., we placed a sample of the diluted homogenate in nutrient broth made with 11.5 g of Difco nutrient agar (Becton Dickinson) and 500 mL distilled water and incubated at 25°C, 30°C, and 35°C over 2 days. We then inoculated this material on agar for all samples that did not initially grow *Wohlfahrtiimonas* spp.

#### **Pulsed-Field Gel Electrophoresis**

To assess relatedness, we performed pulsed-field gel electrophoresis (PFGE) on the isolates using CDC's standardized PFGE PulseNet protocol for *Escherichia coli* O157:H7 (12). Because PFGE has not been previously described for *Wohlfahrtiimonas* spp., we experimented with restriction enzymes commonly used on gram-negative organisms related to *Wohlfahrtiimonas* spp. (13,14), including *NotI* (Roche), *XbaI* (Roche) and *SpeI* (Roche) restriction enzymes. The standard lanes consisted of *Salmonella* ser. Braenderup H9812 cut with restriction enzyme *XbaI*. Gel electrophoresis was run by using CHEF Mapper XA system (Bio-Rad Laboratories, Hercules, CA, USA) for 18.5 hours with initial and final switch time at 2.2 and 54.2 seconds, respectively. Analysis of PFGE patterns was conducted with BioNumerics (version 6.6). *Salmonella* ser. Braenderup H9812 was used as the reference standard.

## Three Results

### Case Investigation and Entomological Identification

On September 15, 2016, we inspected the patient's home, which consisted of an urban, single-story detached building in insalubrious condition. The outdoor temperature range was 21°C–27°C. One window was ajar by ≈5cm, allowing insects to enter the home.

We found a substantial number of dead insects in a desiccated state inside the home. We identified 6 species of flies, including *Calliphora vicina* (blue bottle fly), *Lucilia sericata* (green bottle fly), *Musca domestica* (house fly), *Hydrotaea leucostoma* (garbage fly), *Protophormia terranova* (northern blow fly) and *Fannia* species (lesser house fly). Live larvae (n ≈ 20) were collected from underneath a carpet soiled with organic matter at the exact location where the patient was found. Half of these larvae were kept to perform entomological bacteriology; the other half were left to pupate and emerged as *C. vicina*, *M. domestica*, and 1 unidentified species in the Calliphoridae family (Table). One *L. sericata* (Figure 1) and one *M. domestica* were caught alive in the house and kept in separate sterile containers. Both died during transport, but the *L. sericata* specimen laid eggs inside the container before dying.

### Diagnostic Identification

The patient isolate grew on blood agar, yielding colonies with a smooth center and rough edges and displaying α hemolysis. With time, the colonies spread and exhibited a distinct brown pigment underneath. A brown pigment was also exhibited on heart infusion tyrosine agar. Moderate growth was recovered on MacConkey and phenylethyl alcohol agar. Optimal growth temperatures were 25°C and 35°C in aerobic conditions. No growth was recovered anaerobically or at 42°C. Colonies were strong oxidase positive, catalase positive. Acid was not produced from glucose, D-xylose, mannitol, lactose, sucrose, or maltose. Cells were nonmotile. Gram staining revealed large, pleomorphic, gram-negative rods, including curved and eye-shaped rods, some of which displayed vacuolation.

MALDI-TOF MS recorded a value of 2.40 for *W. chitiniclastica*, but the identification was presumptive because the MALDI-TOF MS database includes only *W. chitiniclastica* and the uniqueness of the isolate required confirmatory 16S rRNA sequencing.



## Entomological Bacteriology

Six insect samples were inoculated on agar plates (Table, samples 2–7). *Wohlfahrtiimonas* sp. was isolated from 2 insect samples on blood agar plates incubated at 25°C. The first isolate was obtained from the fifth wash and diluted homogenate of the larva that hatched from the eggs laid by the green bottle fly. MALDI-TOF MS recorded a value of 2.41 and 2.43, respectively, using RUO library (unvalidated result). The second isolate was obtained from diluted homogenate of the pooled larvae collected from the carpet with a MALDI-TOF MS score of 2.29 (unvalidated result). These larvae were not taxonomically identified. The negative washes from these same samples demonstrated considerable bacterial overgrowth from which *Wohlfahrtiimonas* sp. could not be identified. *Wohlfahrtiimonas* sp. was not isolated from any other samples, including adult flies that emerged from the positive batch of larvae and from the fruit and meat fed to larvae and adult flies.

## Isolate Relatedness

All isolates were successfully cut with *NotI* restriction enzyme. The PFGE pattern indicated that all isolates from flies and fly larvae were indistinguishable and were 74% similar to that of the patient isolate (Technical Appendix Figure 1).

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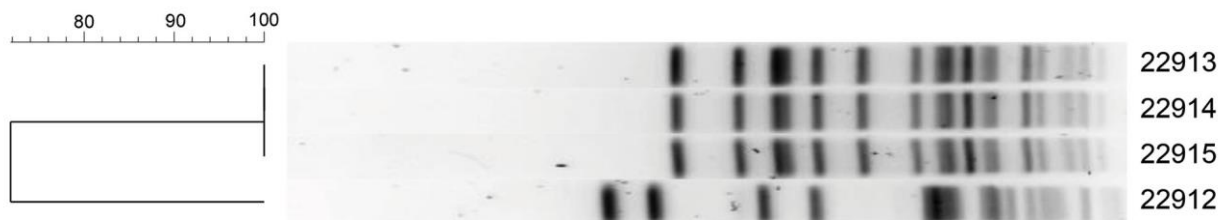
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**Technical Appendix Table.** Summary of reported cases of *Wohlfahrtiimonas chitiniclastica* and the closely related *Ignatzschineria* species (syn. *Schineria* species) isolated among mammals worldwide

Species	Notable clinical presentation or postmortem findings	Organism isolated	Location sampled	Larvae present	Country	Reference
Human	Nonhealing, necrotic ulcers	<i>I. indica</i>	Blood	Yes, species unspecified	United States	(14)
Human	Nonhealing ulcers, urinary tract infection	<i>I. indica</i>	Urine	None	United States	(14)
Human	Severe lacerations	<i>I. indica</i> *	Blood	Third instar larvae of <i>Lucila</i> ( <i>Phaenicia</i> ) <i>sericata</i>	United States	(14)
Human	Shoulder wound, hypotension with auricular fibrillation, cardiorespiratory arrest	<i>I. ureiclastica</i>	Blood	Yes, species unspecified	France	(15)
Human	Maceration of feet	<i>Schineria</i> larvae (syn. <i>I. larvae</i> )	Blood (only bacteria isolated)	Yes, species unspecified	France	(16)
Human	Maceration of feet (sale case as above, reinfection)	<i>S. larvae</i> * (syn. <i>I. larvae</i> )	Blood	Yes, species unspecified	France	(16)
Human	Cutaneous ulcers, fever	<i>S. larvae</i> (syn. <i>I. larvae</i> )	Blood	Yes, species unspecified	France	(17)
Zebra	Unspecified	<i>W. chitiniclastica</i>	Pancreas	Unspecified	China	(18)
Dolphin	Found dead, endocarditis, septicemia	<i>W. chitiniclastica</i>	Blood, cardiac lesions	Unknown	Canary Islands	(19)
White-tailed deer	Found dead, tongue necrosis, septicemia	<i>W. chitiniclastica</i>	Tongue, liver	Unknown	United States (Michigan)	(20)
Cow	Cellulitis, purulence	<i>W. chitiniclastica</i>	Hoof crown	Unknown	China	(21)
Human	Deep ulcer, cellulitis, osteomyelitis	<i>W. chitiniclastica</i>	Ulcer	Unknown	India (Kerala)	(22)
Human	Superficial ulcers, neutropenia, febrile	<i>W. chitiniclastica</i> *	Blood	Yes, not identified	France	(23)
Human	Umbilical and foot wounds, septic shock	<i>W. chitiniclastica</i>	Blood	Yes, not identified	United States (Hawaii)	(24)
Human	Decubitus ulcers	<i>W. chitiniclastica</i>	Deep wound	None	United States (Hawaii)	(24)
Human	Gangrene of distal leg	<i>W. chitiniclastica</i>	Surgically resected bone	None	Estonia	(25)
Human	Superficial ulcers	<i>W. chitiniclastica</i>	Wound swab	None	United States	(26)
Human	Skin excoriation	<i>W. chitiniclastica</i>	Blood	Third instar larvae of <i>Lucila</i> ( <i>Phaenicia</i> ) <i>sericata</i>	United Kingdom	(27)
Human	Occlusive peripheral arteriopathy of lower limbs, probable septic shock	<i>W. chitiniclastica</i> *	Blood	None	Argentina	(28)
Human	Ulcers	<i>W. chitiniclastica</i>	Ulcer	None	Germany	(29)
Human	Ulcers	<i>W. chitiniclastica</i>	Ulcer	None	Germany	(29)
Human	Ulcers	<i>W. chitiniclastica</i>	Ulcer	None	Germany	(29)
Human	Leg ulcer	<i>W. chitiniclastica</i>	Ulcer	None	Germany	(29)

\*Blood samples for which *W. chitiniclastica* or *Ignatzschineria* spp. were the only organisms isolated.



**Technical Appendix Figure.** Dendrogram of pulsed-field gel electrophoresis patterns of isolates from flies and fly larvae, Washington, USA. From top to bottom: batch of larvae (diluted homogenate); *Lucila sericata* larvae (fifth wash); *L. sericata* larvae (diluted homogenate); and patient isolate.